AN INVESTIGATION OF *LOBELIA GIBBOSA* AND *LOBELIA DENTATA.* I.

MYCORRHIZA, LATEX SYSTEM AND GENERAL BIOLOGY.

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(Forty-four Text-figures.)

[Read 25th November, 1931.]

Introduction.—The mature plant.—The seedling stage.—The root system and mycorrhiza.—The shoot system.—The latex system.—General biology, experiments and observations.—Discussion.

Introduction.

The investigation here reported was commenced in Adelaide early in 1916. Miss A. Rennie, working under the direction of Professor Osborn, began an examination of *Lobelia gibbosa* Labill., and made a study of its latex system and a preliminary examination of the fungus present in its roots. She also made a number of field notes on the growth habits of the plant, and some experiments. This work was not published.

In the summer of 1929 Professor Osborn noticed that *Lobelia dentata* Cav. growing in the Sydney district had a number of interesting and peculiar features in common with *Lobelia gibbosa*; and on his advice an investigation was made of both species, and the work previously done by Miss Rennie was confirmed and extended.

The present communication includes the results of both investigations, Miss Rennie having generously handed over her notes, drawings and preparations to the author, who wishes to acknowledge her indebtedness for them.

Text-figures 37–40 are from drawings made by Miss Rennie. The author alone is responsible for any opinions expressed.

The Mature Plant.

Lobelia gibbosa Labill. is reported for all the Australian States, including Tasmania. In South Australia, where part of the work was carried out, it is reported by Black (1922) as occurring on Kangaroo Island, Yorke and Eyre Peninsulas and probably in the south-west. The material for the present work was collected in the scrub at Mt. Lofty, near Adelaide.

Bailey (1900) and Ewart (1930) distinguish three varieties of this species of *Lobelia*, the one here described corresponding to var. *microsperma*, but Black (1922) gives *microsperma* as a synonym for *gibbosa* and distinguishes no varieties. Ewart considers that the species is composed of a complex of hybrids.

L. gibbosa is an annual herb, growing in both sandy and clay soil, often in colonies. The stem is erect and usually unbranched, except sometimes in the region of the inflorescence. It is somewhat turgid and succulent, with a length underground of 2–5 cm., occasionally more. The leaves are all cauline and vary with the habitat, from filiform or linear in poor or exposed situations to narrow lanceolate in sheltered positions, and are somewhat fleshy. The inflorescence is a scorpioid cyme, resembling a terminal one-sided raceme, bearing numerous crowded china-blue flowers which conform to the general *Lobelia* type. Latex is present throughout the whole of the plant, and is especially abundant in the above-ground parts.

The height of the plant was found to vary very much with its habitat, the average is about 47 cm., but in exposed situations it may not be more than 10 cm. If germination takes place early, or in a sheltered position, a tall fleshy stem bearing well developed lanceolate leaves is formed, whereas if germination is late or the plant is in an exposed position, the stem of the mature plant is much shorter and the leaves much narrower. The quantity of anthocyanin pigment developed in such exposed plants is usually quite high, often giving the whole plant a distinct reddish tinge.

The underground part of the stem is white, fleshy and brittle. The roots are whitish when young, and very brittle, relatively infrequently branched, and of a very uniform diameter of 1–1·5 mm.

The feature which first attracted attention to this species was the fact that, at the time of flowering, the leaves, roots and lower parts of the stem are withered and practically dead, the root system and underground part of the stem appearing to serve no function beyond that of holding the plant upright in the soil. Another peculiar characteristic is that material collected for herbarium specimens continues to grow in the press, and to open its flowers, often until no buds remain unopened. Bailey (1900) remarked on these unusual features: "Few plants", he wrote, "are as tenacious of life as this species of *Lobelia*. The root is most delicate and only seems of use to the plant up to the time of the first flower opening, after which it appears to die, and only by being fixed in the ground keeps the plant in an upright position. When preparing herbarium specimens of it, they must be scalded before placing in the papers, otherwise they keep elongating and expanding flowers until the plant is thoroughly exhausted, which takes frequently several weeks."

L. gibbosa is rare and sporadic in the Sydney district. Here a rather similar species, *L. dentata* Cav., is common on the sandstone country. It is found all along the coast and main divide of New South Wales and southern Queensland in sandy soil.

L. dentata differs from *L. gibbosa* in the following features: the flowers are larger and deeper blue, on longer pedicels and less crowded on the axis of the inflorescence; the leaves are ovate and more or less deeply incised; the aerial stem is not fleshy, and it does not increase in length, nor do flowers open in herbarium folders.

Unlike *L. gibbosa,* its stem may be quite frequently branched, below or above the ground level, as well as in the flowering region. Frequently, however, it also may have only a single unbranched stem.

In the field *L. dentata* is found growing practically exclusively in sandy loam soil.

The size and general appearance of *L. dentata,* like *L. gibbosa,* are regulated to some extent by its habitat; plants growing in exposed positions are typically smaller than more sheltered plants, and also have a considerable amount of anthocyanin pigment present in their leaves. The length of the underground stem shows more variation in this species than in *L. gibbosa*; sometimes it may be only about 2 cm., but not uncommonly it reaches 15 cm. or even more in length.

In general, the more favourable the position in which a plant is growing, the longer is its root system functional, and the longer does it remain flowering. In some cases plants which had opened more than twenty flowers still had a growing root system, whereas unfavourably situated plants usually were found to have a withering root system even before the first flower was opened. In the field it was often possible to locate plants which had healthy root systems by an examination of their above-ground parts. They were, as a rule, rather succulent in appearance, with deep-green stem and leaves, whereas plants whose root systems were beginning to wither had paler green, often reddish, and curled leaves, and thinner stems.

The root system, even of large plants, was not often found to extend more than 10 cm. around the plant, and for small plants the radius was often not more than 5 cm. The roots are exceedingly brittle and it was found to be extremely difficult to wash out even a small portion of the root system unbroken. The roots are much interwoven, often in tangled bundles, and rather infrequently branched as compared with the roots of most herbaceous dicotyledons. Their diameter is strikingly uniform, about 1·1 or 1·2 mm., except at the growing apex, where they taper to a short point. Even the main root is very little thicker, and frequently cannot be distinguished from its branches. The roots are creamy-brown when old, and opaque white near the apex, unless they have ceased growing, in which case the apex of the root is also creamy-brown in colour.

On two occasions plants were collected which had no chlorophyll at all and were a watery purple colour. One had just appeared above the ground, but the other was nearly 8 cm. tall and fairly robust. Both were collected in sheltered situations.

The Seedling Stage.

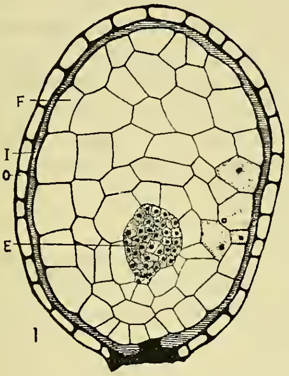
The seeds of both these species of *Lobelia* are exceedingly small. Fifty seeds of *L. dentata* were measured by means of an ocular micrometer and their average length was found to be 0·31 mm., the maximum was 0·39 mm. and the minimum 0·28 mm.; the width varied from 0·21 mm. to 0·29 mm., with an average of 0·25 mm. Their weight is correspondingly small; 100 seeds of *L. dentata* were found to weigh slightly under 1 mgm., so the average weight of a single seed would be slightly less than 0·01 mgm.

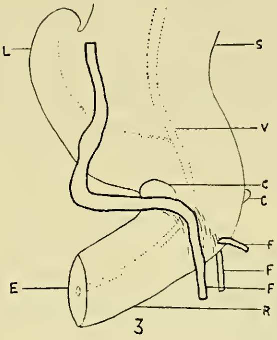
To obtain thin sections of the seeds it was found necessary to adopt the following procedure. The seeds were first soaked in water for a day, they were then fixed in formalin acetic alcohol, washed and placed in 5% caustic potash for seven days; after this they were thoroughly washed in water and then taken up to paraffin. After this treatment sections 6 μ thick could be cut fairly readily.

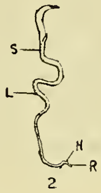
The structure of these minute seeds is very simple; in Text-figure 1 a median longitudinal section is shown. There is a rugose coat of two layers, an outer one (O), a single layer of thick-walled cells, and an inner one (I), which is crushed so that its structure cannot be made out. This encloses an endosperm tissue of fairly large cells, packed with oil. Embedded in this endosperm, near to the micropylar end, is an embryo (E), which consists of much smaller cells and is totally undifferentiated into plumule and radicle.

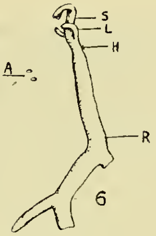
As yet germination of the seeds in the laboratory has not been accomplished. In nature the wastage of seeds must be great. In most years *L. dentata* is locally

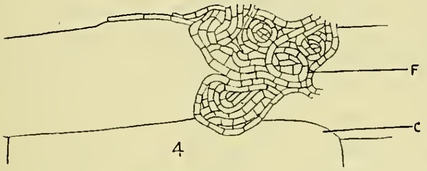
though exceedingly brittle, each individual fragment of root is easily freed from particles of soil since there are no root hairs developed at any time.

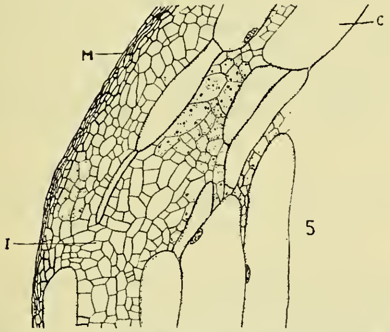


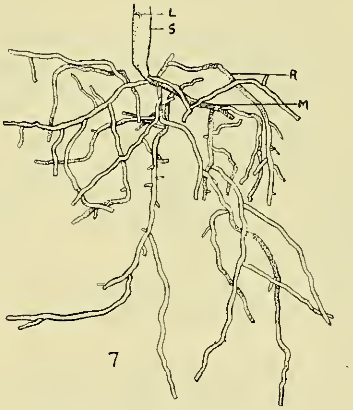












Text-fig. 1.—A longitudinal section of a mature seed of *Lobelia dentata*. O, outer layer of testa; I, inner layer of testa; F, endosperm; E, embryo. × 140.

Text-fig. 2.—A subterranean seedling of *L. dentata*. S, shoot; L, rudimentary leaf; H, hypocotyl; R, root. × 1·1.

Text-fig. 3.—An enlargement of the hypocotyl and part of the root and shoot of the seedling shown in Text-fig. 2. S, shoot; L, rudimentary leaf; R, root; C, rudimentary cotyledons; E, broken end of root; V, vascular strand; F, fungal strands. × 26·5.

Text-fig. 4.—Part of the hypocotyl area of a seedling of *L. dentata*. F, fungus mat over the surface; C, transversely elongated cortical cells. × 250.

Text-fig. 5.—Part of a transverse section of the hypocotyl of a seedling of *L. dentata*. C, cells of the cortex; M, external mat of fungal hyphae; I, invading fungal hyphae. × 250.

Text-fig. 6.—A subterranean seedling of *L. gibbosa*. R, root; S, shoot; L, rudimentary leaf; H, hypocotyl; A, seeds of *L. gibbosa* drawn to the same scale. × 3·5.

Text-fig. 7.—Part of the root system of a fairly large plant of *L. dentata*. S, base of underground stem with rudimentary leaves (L); M, main root; R, lateral roots. × 0·67.

The smallest seedling found of *L. gibbosa* is shown in Text-figure 6; it is smaller than any of the seedlings found of *L. dentata*, and was discovered by accident during the washing out of the root system of a larger plant. It shows indications of a fairly large root system (R), but the shoot is still rudimentary. Fortunately Miss Rennie, who discovered the specimen, mounted it complete in glycerine jelly, and on examination of the hypocotyl region a structure was observed quite similar to that shown by the seedling of *L. dentata* in this area. There is a region of transversely elongated cells, the surface of which is covered by a fungus mat, and there is also an associated strand of fungal hyphae.

Root System and Mycorrhiza.

Text-figure 7 shows part of the root system of a plant of *L. dentata* washed out from sandy soil; it represents less than half of the total number of roots and few of the branches are intact, but it brings out clearly the uniform diameter of the roots, and the method and frequency of branching.

The internal structure of the roots is quite simple; the lateral roots have invariably a diarch xylem, but in the main root the xylem is usually 3–4-arch, latex vessels are present in the phloem in the position of sieve tubes, the endodermis is unthickened and the cortex fairly wide. Secondary thickening takes place only in old roots and new elements are added to both xylem and phloem.

The size attained by subterranean seedlings as compared with the size of the seeds from which they develop is very striking. The seedling illustrated in Text-figure 6 was the smallest found, but it is relatively enormous in comparison with the two seeds (A) drawn to scale beside it. On account of this great difference in size between the seed and the colourless seedling resulting from it, it was suspected from the beginning of the investigation that the species of *Lobelia* under discussion were mycorrhizal. Miss Rennie succeeded in demonstrating the presence of a fungus in the roots of *L. gibbosa*, but did not make a detailed examination of it.

This has now been done for *L. dentata* and confirmed for *L. gibbosa*, and there is every reason for believing that the two species are identical in this respect.

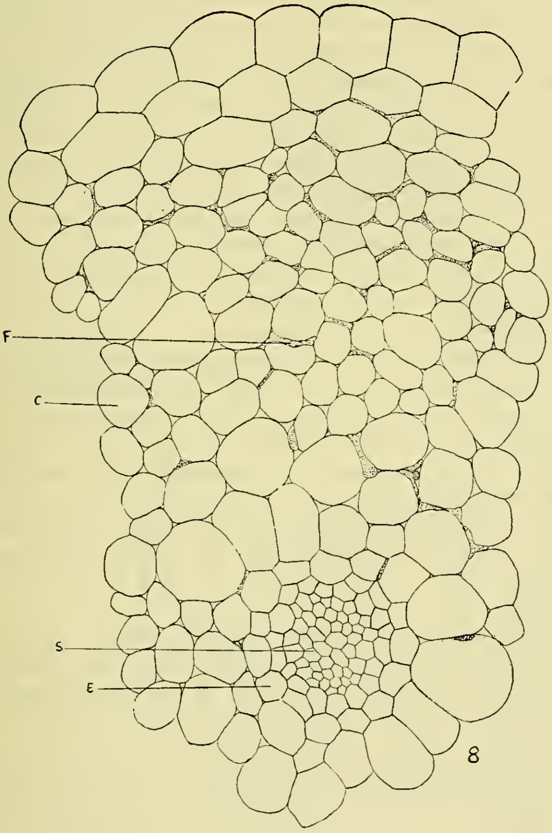
The presence of a fungus in the root tissues of *Lobelia* is readily demonstrated, but the amount found in various sections and the condition in which it was present, were found to be very variable, and seemed to conform to no rule. In some cases only a trace of mycelium was present in the outer part of the cortex; in other cases it was found to be more generally distributed. Finally it was found necessary to fix the material in the field, and to choose long complete pieces of root, cutting sections at intervals from the apex to the older parts.

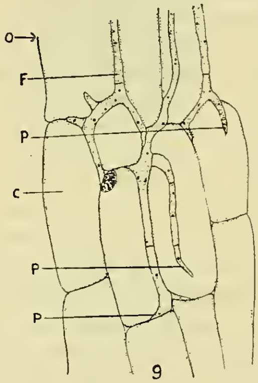
It was found that when material is brought into the laboratory for examination in the living condition, changes often, though not invariably, take place, principally the total disappearance of all reserve food from the fungus, thus giving an entirely wrong impression of the condition of the fungus within the root as growing in the field. Sections of roots which are not actively growing also tend to be misleading.

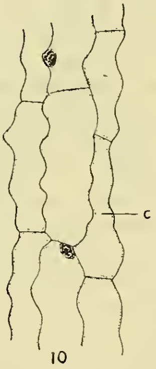
Flemming's weak fixative was found to be best for roots and young stems, and its fat staining property was very useful in the case of young roots. Sections were stained with Gentian Violet and Orange G, or if, as was the case for young roots, much oily reserve food were present, the sections were simply stained with Orange G or Erythrosin; material fixed in chromo-acetic fixative was best stained by the iron alum haematoxylin method.

The development of the fungus within the root.—The following description and figures of the development of the fungus within the root are of *L. dentata*; *L. gibbosa* is substantially the same and will not be separately described.

Text-figure 8 shows part of a transverse section of a growing root taken about 7 mm. from the apex. The cells of the stelar region (S) are as yet practically undifferentiated; the cells of the cortex are fairly large and there is no sign of root hairs. In the cortex, fungal hyphae (F) can be readily distinguished; they are strictly intercellular, and though many sections were examined, no sign of







Text-fig. 8.—Part of a transverse section of a young root showing the stage of fungal invasion. S, undifferentiated stele; E, endodermis; C, cells of the cortex of the root; F, fungal hyphae. × 250.

Text-fig. 9.—Part of a radial longitudinal section of the middle cortex of young root showing the fungal hyphae growing down between the cells of the cortex. C, cortical cells; F, fungus; O, older part of root; P, pointed growing apices of the fungus. This was drawn from a section which was cut rather thick for the purpose of examining the growing points of the fungus. Those hyphae, therefore, which appear to be penetrating into the cell cavity are in reality outside the cell wall. × 250.

Text-fig. 10.—Part of a tangential longitudinal section of the outer cortex of a very young root showing cortical cells (C) with sinuous radial walls. × 250.

haustorial organs could be discovered. A longitudinal section in this region (Text-figure 9) shows that the fungal hyphae are growing down from the older part of the root. They are fairly narrow and densely protoplasmic, with rather pointed growing apices (P), and they force their way down between the cells of the middle cortex. This may be called the region of fungal invasion.

In a tangential section very near the apex of a growing root, and above the fungus, the outer cells of the cortex are seen to have curiously sinuous radial longitudinal walls (Text-figure 10); this curvature becomes more and more pronounced further from the apex. It becomes still more marked when the fungus grows down between the cells, and the penetrating fungal hyphae have a correspondingly sinuous course (F in Text-figure 11). In a radial section the walls of these cells are often cut so that they resemble a string of small cells end to end, sometimes with patches of mycelium between them (C in Text-figure 14). As seen in the radial longitudinal section shown in Text-figure 14, the tangential walls of these cells are occasionally slightly sinuous also (S). It is only the outer two or three layers of cortical cells which show this feature. The inner cortical cells at first have quite straight longitudinal walls (Text-figure 9), though they later become somewhat distorted by the intercellular growth of the fungus. There is no possibility of this phenomenon being the result of faulty fixation or embedding, since it was observed in all longitudinal sections cut, and in preparations of living roots. It proved to be a feature of some importance in the identification of small subterranean *Lobelia* seedlings.

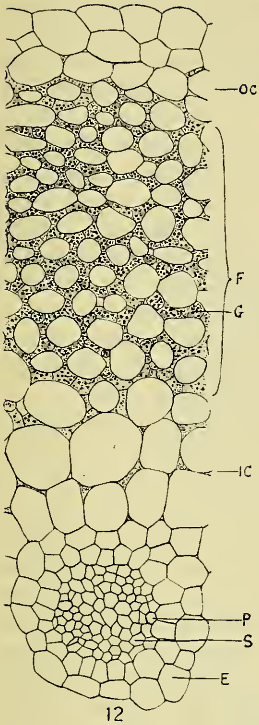
A section taken about 3 cm. from the root apex shows the next stage in the development of the mycorrhiza (Text-figure 12). This may be called the period of fungal enlargement. In a region which may be called the fungal zone, limited in most cases to the middle cortex, there has been a great concentration in the amount of fungal tissue present. The individual hyphae are much wider than in the region of fungal invasion, and a good deal of branching has taken place. The cortical cells are often forced apart, and in transverse section appear as practically isolated islands in the mass of fungal tissue. At this time the fungal hyphae stain much more darkly with osmic acid than they do in the region of fungal invasion. This is due to the presence in them of small darkly staining droplets of reserve food material.

A tangential section of the middle cortex at this stage (Text-figure 13) shows the fungal hyphae forming a network between the cortical cells, branching and anastomosing in all directions. Text-figure 14 shows part of a radial longitudinal section of a root at a slightly earlier stage than that shown in Text-figure 12;

Text-fig. 14.—Part of a radial longitudinal section of a young growing root during the period of fungal enlargement. E, endodermis; I.C, inner cortex; O.C, outer cortex; F, fungal zone; C, the cut edges of the walls of the sinuous walled cells of the outer cortex; S, slightly sinuous tangential walls of the cells of the outer cortex. × 150.

Text-fig. 15.—Part of a radial longitudinal section of a young growing root showing the final development of the fungus during the period of fungal enlargement. It shows the large size of the droplets of reserve food in the hyphae of the fungal zone as compared with those in the hyphae of the inner and outer cortex, and the great enlargement of the fungal hyphae in the fungal zone. O.C., outer cortex; I.C., inner cortex; f, fungal zone; D, droplets of reserve food; E, endodermis; F, fungal hyphae. × 270.

Text-fig. 16.—Part of a tangential section of a young growing root in the fungal zone showing a dense network of fungal hyphae with large accumulations of reserve food. F, fungal hyphae; D, droplets of reserve food. × 435.



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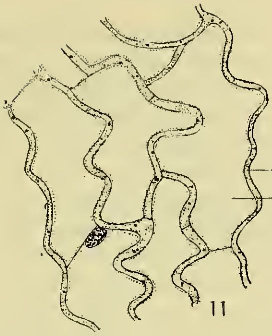

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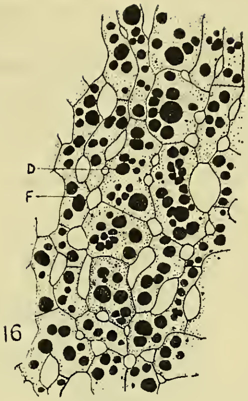

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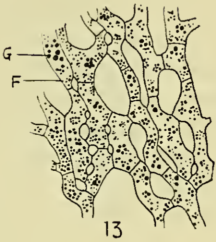

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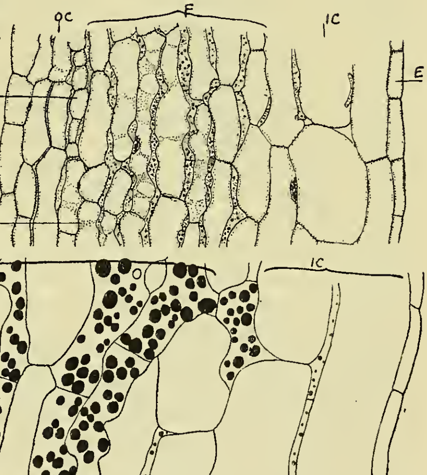

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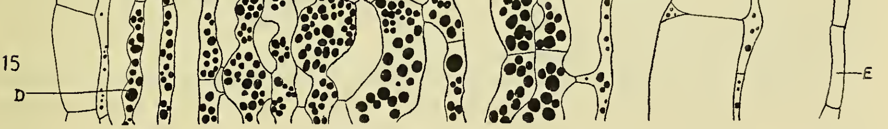

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Text-fig. 11.—Part of a tangential longitudinal section of the outer cortex of a slightly older root than that shown in Text-fig. 10, showing that the radial walls are even more sinuous, and that the fungal hyphae between them are correspondingly sinuous. C, cortical cells; F, fungal hyphae. × 270.

Text-fig. 12.—Part of a transverse section of a young growing root showing the commencement of the period of fungal enlargement. S, stele; P, protoxylem; E, endodermis; I.C, inner cortex; O.C, outer cortex; F, fungal zone; G, small droplets of reserve food accumulating in the hyphae of the fungal zone. × 150.

Text-fig. 13.—Part of a tangential longitudinal section of a young growing root in the fungal zone during the period of fungal enlargement, to show the formation of a network of hyphae between the cells of the cortex. F, fungal hyphae; G, droplets of reserve food. × 270.

concentration of the mycelium has begun in the fungal zone (F), and small droplets of reserve food material are present. Both longitudinal and transverse sections fail to show any signs of the development of fungal haustoria into the root cells. These cells appear to be perfectly healthy, the nucleus shows no sign of distortion and is usually somewhat fusiform, cytoplasm lines the cell wall, but there is no sign of reserve food (Text-figs. 8, 14).

Text-figures 15 and 16 show respectively radial and tangential longitudinal sections of a root showing the maximum development of the fungus in the fungal enlargement stage. In Text-figure 15 the differentiation of the cortex into three zones, outer and inner cortex and fungal zone, is clearly brought out. The fungus in the outer and inner cortex has much less reserve food than the hyphae in the middle cortex. Here the fungal hyphae are much extended by the presence of the reserve food which has accumulated in much larger droplets than elsewhere. Text-figure 16 shows a small part of a tangential section taken in the fungal zone; the mycelium forms a practically complete network and shows the great distortion caused by the accumulation of reserve food in its cells.

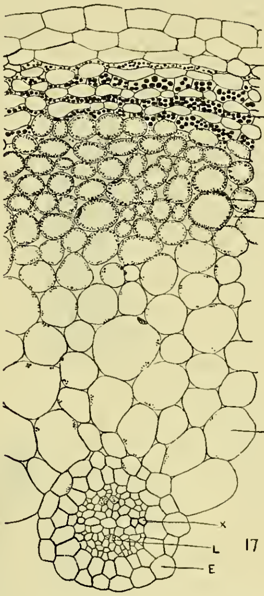
The third stage of the mycorrhiza may be called the period of fungus depletion, and its progress is shown in Text-figures 17 and 19, which were taken respectively 4 cm. and about 6 cm. from the apex of an actively growing root. It will be seen at once that the cells of the inner cortex are much enlarged and there is scarcely any trace of fungal mycelium between them. Important changes are also evident in the inner part of the fungal zone; the cortical cells in this region have enlarged somewhat, and in the cytoplasm lining their walls are numerous, small droplets of a reserve food similar in staining properties to that of the fungal hyphae. Between these cells the hyphae appear somewhat compressed and have lost practically all their reserve food. Depletion of the fungus is most marked in the innermost part of the fungal zone, and the hyphae in the inner cortex are so crushed as to be practically invisible. The process of the depletion of the fungal hyphae is shown in greater detail in Text-figure 18. At A the fungal hyphae are much distended by the presence in them of large accumulations of reserve food, and the cortical cells between are rather distorted, but otherwise appear quite healthy; there is no sign of fungal haustoria. At B droplets of reserve food are beginning to appear in the peripheral cytoplasm of the cortical cells and the fungal hyphae between them are slightly narrower than at A, and have less reserve food. At C one can see that the fungus has lost all reserve food, and though still densely protoplasmic, is being gradually crushed by the expansion of the cortical cells. The nuclei of the cortical cells remain quite unaltered in shape and size during this period.

The process of depletion of the fungal hyphae and engorgement of the cortical cells goes on progressively towards the outer cortex. Its progress may be traced in sections cut further and further from the apex of the root till finally all reserve food is seen to have disappeared from the fungus; during this period the cortical cells extend and the hyphae between them are so compressed that they are hard to detect. Concurrently with this change the latex vessels in the phloem come to contain material which stains darkly with osmic acid (Text-figs. 17, 19); from this it appears that the food material absorbed from the fungus by the cortical cells is transported away at once.

In older roots no sign of the fungus can be detected except in the outermost region of the cortex where a few threads of it persist, apparently in the living condition. Text-figure 19 shows the final stage of the fungus depletion period.

A few drops of reserve food are still present in the outermost cells of the fungal network (D), and the latex vessels are densely staining, evidently in the process of transporting away food materials.

Throughout this description it has been emphasized that these are the changes which occur in an actively growing root. In roots which are growing slowly, the changes described may be telescoped into a much shorter length of root, and in the sections cut of roots which had ceased to grow, it was seen that the fungal hyphae were entirely devoid of food materials.



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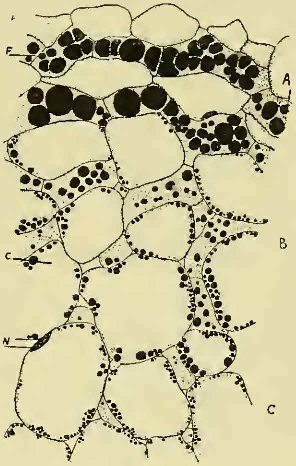

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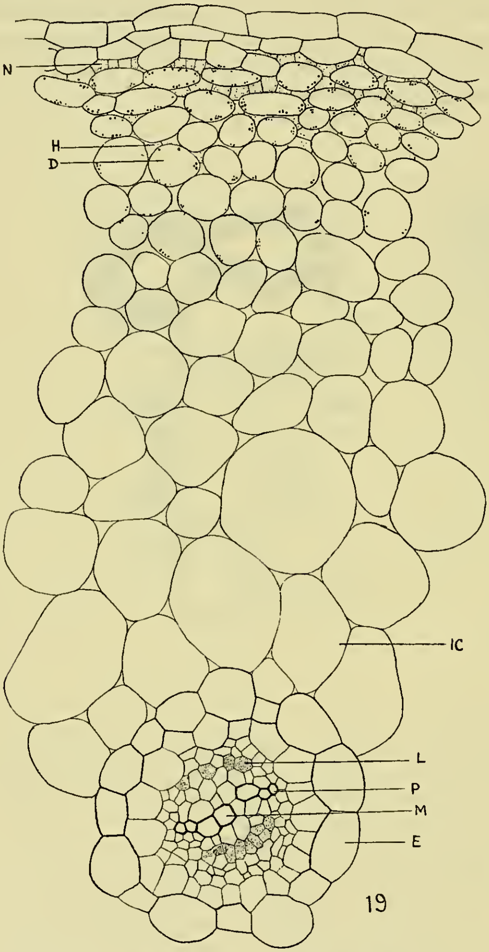
Text-fig. 17.—Part of a transverse section of a young growing root showing the commencement of the period of fungal depletion. X, protoxylem; L, latex vessels; E, endodermis; I.C, inner cortex; O.C, outer cortex; D, cortical cells of inner fungal zone showing accumulations of droplets of reserve food in their peripheral cytoplasm; H, depleted fungal hyphae between these cells. U, unaltered hyphae in outer fungal zone.

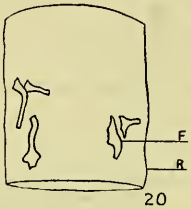
Text-fig. 18.—Part of the section shown in Text-fig. 17 showing in greater detail the process of depletion of the fungal hyphae. At A the fungal hyphae are unaltered and the cortical cells between them are devoid of food material. At B some of the reserve food has gone from the hyphae and droplets are appearing in the peripheral cytoplasm of the neighbouring cells. At C all reserve food has gone from the fungus. F, fungal hyphae; N, nucleus of cortical cell; C, cortical cell. × 300.

As was previously stated, it was found that the colour of the root is a reliable guide to its internal condition; clean opaque white roots are in the fungus enlargement condition, creamy roots are in the fungus depletion condition, and the fungus within brownish-cream roots is entirely depleted of food material. Roots with brownish-cream tips have ceased growth, and sections taken of these show no sign of the presence of reserve food material.

Occasionally roots have been found which are rather thinner than usual, and nearly hyaline. These were always associated with plants which had developed an aerial shoot. When sections of these were examined it was found that they showed no trace of the fungus, and also lacked sinuous-walled cells in the outer cortex. A similar kind of root developed when plants brought in from the field were planted in pots and allowed to grow in a glasshouse under good conditions.



Text-fig. 19.—Part of a transverse section of a young growing root showing the final stage of the period of fungal depletion. P, protoxylem; M, metaxylem; L, latex vessels; E. endodermis; I.C, inner cortex; D, cortical cells in the outer part of the fungal zone with few peripheral droplets of reserve food; H, depleted and crushed hyphae between them; N, living and uncrushed, but depleted fungal hyphae in the outer cortex. × 150.



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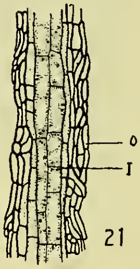
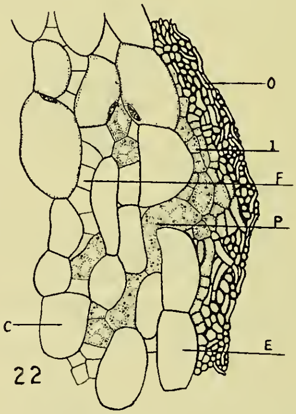
Text-fig. 20.—Part of a root of *L. dentata* showing a number of associated strands of fungal hyphae (F). × 14.



Text-fig. 21.—A longitudinal section of a fungal rhizomorph. O, outer layer of hyphae; I, inner hyphae. × 270.



Text-fig. 22.—Part of a transverse section of a root of *L. dentata* passing through the point of entry of a strand of fungal hyphae. O, outer layer of hyphae; I, inner hyphae; P, invading strand; E, epidermis; C, cells of the cortex of the root. × 270.

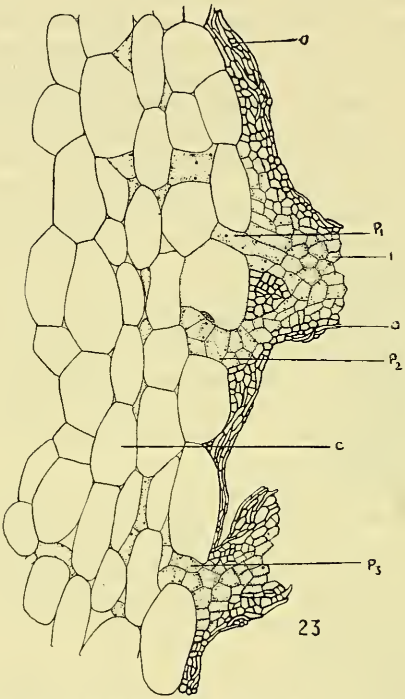
On one occasion three plants from the same locality were examined and it was found that they had developed roots which were slightly narrower than the typical roots and, though white near the apex, were not quite opaque. These roots were evidently in a condition of active growth, and an intercellular mycelium was found to be present, which invaded newly-formed tissues by growing downwards from the older part of the root. After invasion the fungus increased in amount in the inner cortex and subsequently became crushed out, just as is the case in a typical root. These roots differ from the typical roots in that they show little or no sign of the accumulation of a reserve food in the intercellular fungus nor of the transference of food materials from the fungus to the cells of the cortex of the root. On the occasion when these plants were found other plants were collected in the same locality whose roots showed the typical accumulation of oily reserve food in the intercellular fungus, and the subsequent transference of this to the cells of the cortex of the root.

The invasion of the root by fungus strands growing in the soil.—Although numerous sections of roots were examined, no trace of an external mycelium could be discovered until pieces of root were examined whole under the microscope. It was then immediately found that associated with the roots were very narrow rhizomorph-like strands of fungal hyphae similar to those found in connection with the hypocotyl of seedlings. As a rule these did not spread out very far over the surface of the root (Text-fig. 20).

The number of fungus strands per unit area of root is very variable. Sometimes a large number are found close together, as shown in Text-figure 20, at other times lengths of as much as 5 cm. of root were examined and not a single fungus strand was discovered. The fungus strands are brown and brittle, breaking off close to the root very easily. Generally they are most abundant on the older parts of the roots, and only very rarely were any found in association with the first 2–3 cm. of a growing root.

The fungus rhizomorph resembles the mat of fungal hyphae covering the hypocotyl in that it consists of two parts, an outer and an inner (Text-fig. 21). The outer layer (O) consists, as in the fungus mat, of thick-walled, narrow hyphae, running in a general longitudinal direction, but in places much twisted. This forms a continuous tissue over the core of the strand, which consists of one or more longitudinally running hyphae (I) which are much wider than those of the outer tissue, and are thin-walled, densely protoplasmic and multinucleate.

When one of these strands grows into contact with a *Lobelia* root, the thick-walled hyphae of the outer layer spread out over the surface of the cortex, forming a mat which may be extensive or, more usually, is quite small. The cells of the core come into direct contact with the outer cells of the root. From this core a strand of the fungus, usually consisting of a single hypha, more rarely of two or more, grows down between two epidermal cells and into the cortex of the root crushing out the hyphae of the fungus which had grown down within the root from the hypocotyl. Text-figure 22 is part of a transverse section of a root showing the outer cortex and an associated strand of fungal hyphae which is cut obliquely. A single penetrating hypha (P) has grown down from the strand into the cortex of the root. Between the cells of the cortex two kinds of fungal hyphae can be distinguished: one is densely protoplasmic and evidently is connected with the outer hyphal strand; the other (F), the hyphae previously present in the root, has very little protoplasm. In Text-figure 23 a more complex and less usual condition is shown. Above a branched strand is penetrating the root in two places, P1 and P2; P2 is a complex structure consisting of several hyphae. Below another fungal strand is present which also has a compound penetrating strand, P3.



Text-fig. 23.—Part of a transverse section of a root of *L. dentata* showing the entry of several strands of fungal hyphae. O, outer layer of hyphae; I, inner hyphae; P1, P2, P3, invading strands of hyphae; C, cortical cells. × 250.

Except in places where branching took place (Text-fig. 23), only one place of entry into the root could be found for any one fungus strand; it seems, therefore, probable that the growing point of the rhizomorph becomes modified and penetrates into the root rather than that the rhizomorph runs along the surface of the root, having several points of entry.

The Shoot System.

a. External Structure.

Lobelia dentata.—The shoot at first grows much more slowly than the root. The plant illustrated in Text-figure 2 had a comparatively large root system, which unfortunately was damaged when it was being excavated. The shoot increases in diameter, becoming more and more succulent and brittle. It remains almost transparent, but before long a central white strand is visible: this is the stele.

The general habit of the young plant appears to depend largely on the type of soil in which it is growing. In light sandy loam *L. dentata* has usually a single straight shaft, rarely branching except in the flowering zone (Text-figs. 24, 25).

But seedlings were found, when excavating in heavy sandy clay, which showed a rather different habit of growth. Usually they were more succulent and of greater diameter than the first type. They occasionally have a simple unbranched shoot, such as shown in Text-figure 26, but more often the shoot is profusely branched at varying levels below the surface of the soil (Text-fig. 27). Text-figure 27 illustrates an entirely subterranean seedling, and its size, compared with that of the seed from which it originated, is very remarkable. Nearly always these seedlings are gnarled and twisted (Text-figs. 27, 28).

At this stage the latex vessels in the stem are well developed and their contents stain darkly with osmic acid and with iodine, but have not the milky-white appearance of the latex of the mature plant. If a living subterranean shoot be cut across, a little watery white fluid oozes out from the stele, but the greatest amount of bleeding is from the cut cells of the cortex. When the *Lobelia* shoot grows above the surface of the ground, leaves are expanded and become green, and to all appearances the young plant becomes self-supporting. The latex throughout the aerial and underground stem now becomes milky and abundant, and is evidently at much greater pressure within the vessels, since, when a cut is made, a copious flow at once takes place from the region of the phloem. It is perhaps significant that the chlorophyll-lacking plants previously mentioned possessed very little latex, and that this was thinner and more watery than that of the normal plant. This, together with the fact that subterranean seedlings as a rule have no typical latex, suggests that the production of milky latex is in some way bound up with photosynthesis.

A frequent feature of the large seedlings which develop in heavy soil is that the aerial shoot is at first much more slender than the underground part (Text-figs. 26, 30). This is not so noticeable in the young plants which develop in light sandy soil. The length of time elapsing between the first appearance of the plant above ground and the opening of the first flower is entirely variable, as is also the length of the flowering season. Both depend directly on the position in which the plant is growing and to a smaller extent on its initial size before coming above the ground. Plants in favourable situations may take three or four weeks till flowering and continue flowering for a month or more. By "favourable" is meant a moderately shaded position, and moist cool soil. Exposed plants flower more quickly and for a shorter period.

Plants in full flower, which were dug up and brought into the laboratory and abundantly supplied with water, formed new roots to replace those which had been damaged. They continued to flower for 4 to 6 weeks and individual flowers remained open 7 to 10 days. Two plants which had finished flowering in the field, when brought into the laboratory, developed fresh lateral shoots and opened several flowers.

A large underground seedling usually ensures a fairly large mature plant even in dry situations; but of two plants which have an equal underground part, one in a favourable position will almost invariably give rise to a larger plant than one growing under more exposed conditions. On the other hand, a small underground seedling in an exposed situation usually means a small mature plant, sometimes only 5 or 6 cm. tall, with 4 or 5 small leaves and a single flower. But this is by no means the case in favourable positions. In this case quite a slender seedling may ultimately grow into a flourishing plant (see Text-fig. 25, in which the plant illustrated has a very short underground stem).

In the case of dwarfed plants growing in unfavourable positions, it is practically always found that the root system commences to decay before flowering, but in the case of those in favourable situations, actively growing or at least living roots are always present until flowering has commenced, and often afterwards.

In *L. dentata* a method of vegetative reproduction was discovered which proved to be quite common. This was the production of shoots from buds developed on old roots. Text-figure 29 shows a fragment of the root system of a large plant and on it two young shoots have arisen. Text-figure 30 shows a relatively large plant arising adventitiously from a secondary root, quite close to the main plant, which in this case is still quite young.

Before or during flowering the root system and the base of the aerial shoot commence to die. Occasionally this is irregular so that isolated fragments of unwithered root occur between lengths of withered dead root. The decaying root system may become entirely isolated from the shoot, but often a plant can be excavated which shows the dying remnants of its root system still adhering to it. The above-ground parts of the plant represented in Text-figure 31 were practically dead, and most of the root system had decayed away, but two fragments of living root still remain attached to it. In several instances plants whose aerial shoots were quite dead and dry were excavated and in two cases such fragments, though withered at each end, had given rise to small healthy shoots (Text-fig. 32).

Lobelia gibbosa.—As a rule seedlings of *L. gibbosa* (Text-fig. 33) have shorter subterranean stems than do seedlings of *L. dentata*, but their root systems are equally extensive as compared with the size of the shoot. The young plant grows rapidly and at an early stage the aerial stem becomes fairly thick and succulent (Text-fig. 34), becoming more and more so until the inflorescence is produced. Text-figure 35, a plant just before flowering, shows the relatively great thickness of the stem as compared with that of *L. dentata* at a comparable stage of development (cf. Text-fig. 25). The stem begins to dry and die away from the root

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Text-fig. 26.—A young seedling of *L. dentata* from hard sandy clay showing increased diameter and crookedness of the shoot. S, shoot; R, root; L, rudimentary leaf. × 0·44.

Text-fig. 27.—A subterranean seedling of *L. dentata* from hard sandy clay showing twisted branching shoot. S, shoot; R, root; L, rudimentary leaf. × 0·7.

Text-fig. 28.—A subterranean seedling of *L. dentata* from hard sandy clay showing much twisted shoot. S, shoot; R, root; L, rudimentary leaf. × 0·7.

Text-fig. 29.—Young shoots of *L. dentata* arising adventitiously on the root system of a large plant. S, shoot; R, root. × 0·8.

Text-fig. 30.—A large branched plant of *L. dentata* from hard sandy clay soil showing a number of shoots (A) arising adventitiously from the one point on a lateral root (L.R.). S, shoot of the main plant; R, main root of the plant S; GL, ground level. × 0·6.

Text-fig. 31.—The lower part of the stem (S) of an old plant of *L. dentata* showing the decaying root system and fragments of living root (R). L, rudimentary leaf; A, axillary shoot. × 0·72.

Text-fig. 32.—An adventitious shoot (S) arising from an isolated piece of living root (R). × 0·44.

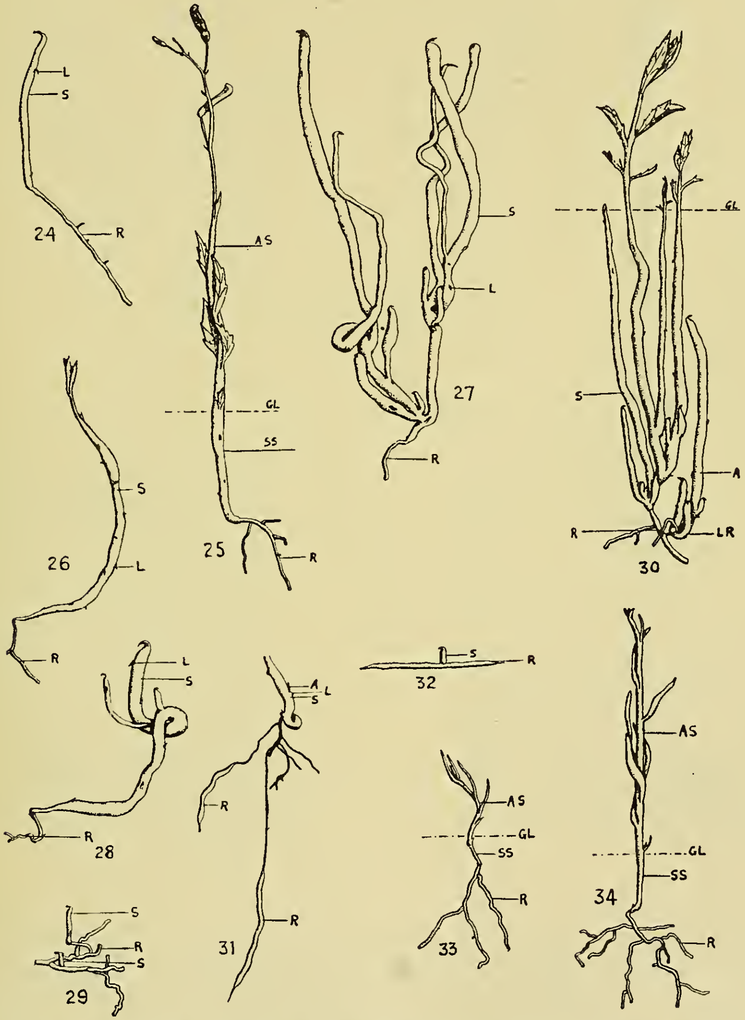
Text-fig. 33.—A young seedling of *L. gibbosa* showing the short length of the underground stem (SS). AS, aerial shoot; R, root; GL, ground level. × 0·4.

Text-fig. 34.—An older plant of *L. gibbosa* showing fleshy aerial stem. AS, aerial stem; SS, subterranean stem; R, root; GL, ground level. × 0·4.

upwards and the leaves wither about the time of flowering, and during the flowering period the aerial stem shrinks gradually in diameter (Text-fig. 36).

b. Internal Anatomy.

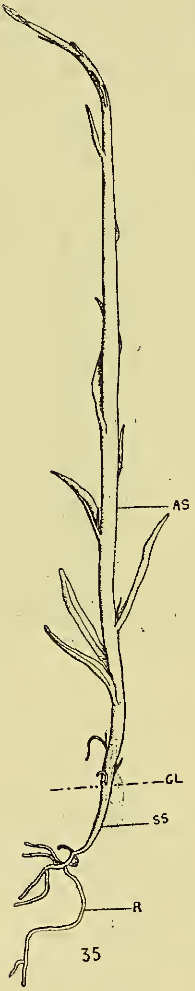
Lobelia dentata.—A transverse section of the shoot of a subterranean seedling shows the structure illustrated in Text-figure 37. The pith which is moderately wide and consists of large rounded cells, is not shown completely. It will be seen at once from an examination of the figure that the cortex (C) is unusually wide, and the cells are very turgid. There are usually a very large number of primary



Text-fig. 24.—A young subterranean seedling of *L. dentata* from light sandy soil. S, shoot; R, root; L, rudimentary leaf. × 0·48.

Text-fig. 25.—A young plant of *L. dentata* from light sandy soil showing the straight unbranched stem. AS, aerial stem; SS, subterranean stem; R, root; GL, ground level. × 0·4.

xylem groups (X) arranged fairly close together in a wide ring, and the phloem (Ph) forms a practically continuous ring outside them. Sieve tubes appear very small in transverse section, and in longitudinal section cannot always be distinguished from the parenchyma. Seedlings which develop in light sandy soil



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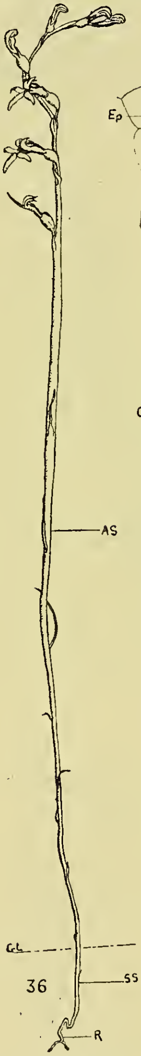

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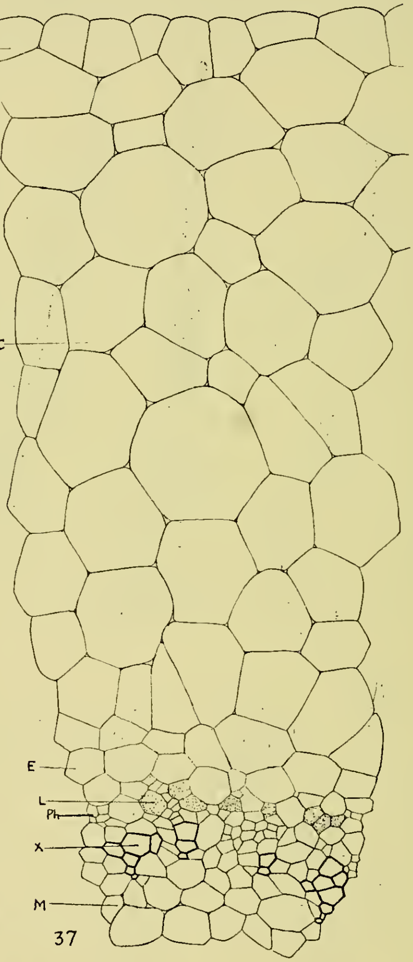

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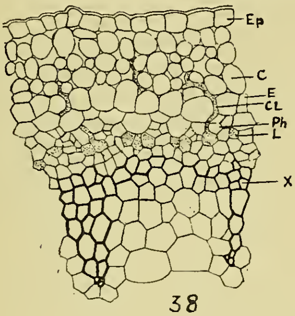
Text-fig. 35.—A plant of *L. gibbosa* just before flowering, showing the great size of the aerial stem (AS) as compared with that of the underground stem (SS). R, root; GL, ground level. × 0·4.

Text-fig. 36.—A flowering plant of *L. gibbosa* showing the withering of the root (R), and the lower part of the stem, and the leaves, and the decrease in thickness of the aerial stem (AS). SS, subterranean stem; GL, ground level. × 0·4.

Text-fig. 37.—Part of a transverse section of a very young underground stem of *L. dentata.* Ep, epidermis; C, cortex of large distended cells; E, endodermis; Ph, phloem; L, latex tubes; X, primary xylem; M, medulla. × 125.

may not have so wide a cortex as figured, but seedlings which develop in hard sandy clay often have an even wider one.

If sections are taken of the young aerial shoot of a seedling which has developed in heavy soil, the reason for the diminished diameter of its aerial stem is at once apparent. Text-figure 38 shows part of a transverse section of a fairly young aerial shoot; the width of the cortex is much less than in the underground stem, both in number and in size of cells, and becomes more so as we proceed upwards, but the diameter of the pith remains fairly constant. The



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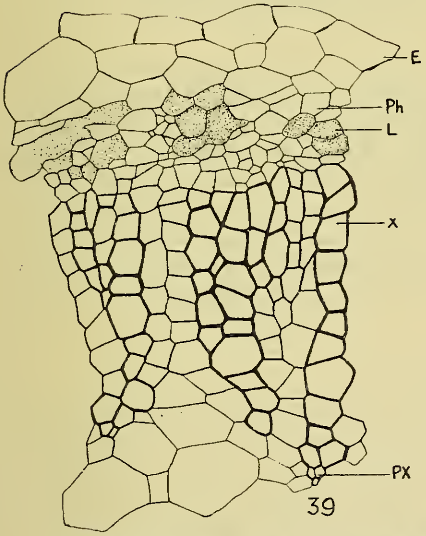

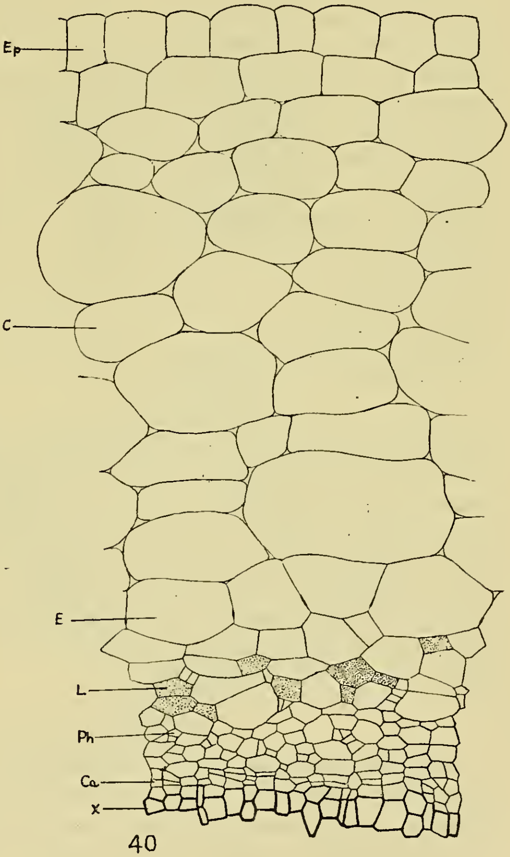

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Text-fig. 38.—Part of a transverse section of a young aerial stem of *L. dentata*. Ep, epidermis; C, cortex; E, endodermis; CL, latex tubes in the cortex; Ph, phloem; L, latex tubes in the phloem; X, xylem. × 150.

Text-fig. 39.—Part of a transverse section of a fairly old underground stem of *L. dentata* showing a small portion of the stele. E, endodermis; Ph, phloem; L, latex tubes; X, secondary xylem; PX, primary xylem. There is no sign of an active cambium, so that secondary growth has evidently ceased. × 150.

Text-fig. 40.—Part of a transverse section of the underground stem of a plant of *L. dentata* which has just begun to flower. Ep, epidermis; C, cortex; E, endodermis; L, latex tubes; Ph, phloem; Ca, cambium; X, secondary xylem. × 150.

activity of the cambium which commences very early in the aerial stem, spreading later to the subterranean parts, soon tends to equalize this difference of diameter. The greatest amount of secondary thickening takes place at the ground level, the amount of secondary xylem diminishing both upwards and downwards so that the stem usually tapers in both directions from the soil level (Text-fig. 25). Secondary xylem consists mainly of fibres and sclerized parenchyma with few vessels. There is little secondary phloem, but in the oldest stems the latex system may be much enlarged (Text-fig. 39).

The underground part of the stem up till the appearance of the first flower bud, or perhaps a little before, is almost transparent, and one can see the stele as a slender white strand in the centre. But with the commencement of secondary growth it becomes less and less so and is finally a uniform opaque white. This may be due to the great shrinking in diameter of the cortex, and to the increase in the amount of the latex. In Text-figure 40 is seen part of a transverse section of a fairly old underground stem which shows the commencement of this shrinkage of the cortex; this shrinkage is certainly not entirely due to the amount of secondary growth taking place in the stele, since at this time only a little secondary wood may have been added. It seems rather to be due to a loss of water from the cortex to the latex system, since, as was previously pointed out, if an old stem be cut across little fluid oozes out from the cortex, but a good flow of latex takes place from the phloem, whereas if a stem such as shown in Text-figure 27 be cut across very little sap oozes out from the phloem, most of it coming from the cut cells of the cortex.

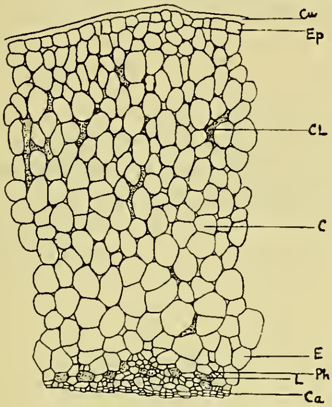
The anatomy of the root and underground stem of *L. gibbosa* is not sufficiently different from that of *L. dentata* to need a separate description. The main difference is in the aerial stem, which, as we have seen, is much thicker and more succulent than in *L. dentata.* A section of a young stem at about the stage shown in Text-figure 32 shows that the reason for this is that the cortex is very wide, wider even than it is in the subterranean part of the stem. Text-figure 41 is part of a transverse section taken in this region, and it can be seen at once that the cells of the cortex are very large and turgid and resemble rather the cells of the cortex of the young subterranean stem of *L. dentata* than those of the young aerial stem.

The Latex System.

The latex system is similar in both species, and will only be briefly described, since Ydrac (1905) has given a fairly full description of the latex system of the Lobelias. Ydrac found that there were two kinds of latex tubes in this genus: (1) The main latex vessels in the phloem, which run more or less vertically and result from the breaking down of end walls between superimposed rows of cells, and are therefore true latex vessels, and (2) branches which originate as outgrowths of these, and form connecting links between the vessels, and also ramify in a winding fashion through the cortex.

There is no essential difference in the latex system of the two species under discussion and that described for the Lobelias by Ydrac, save that there were no latex vessels developed in the pith or xylem at any place. One point noticed by Ydrac is especially conspicuous in these two species: this is that the latex tubes are confined to the phloem in the root and the underground part of the stem, but in the aerial stem branches ramify through the cortex, first having forced their way out from the stele between the cells of the endodermis.

As we have already seen, latex vessels develop early in the young root (Text-fig. 19), and are at first small and few in number; they later enlarge considerably and branch so that a fairly extensive network is formed. When secondary thickening occurs new phloem elements are added, and some of these become latex vessels and link up with the primary latex vessels by means of outgrowths of their



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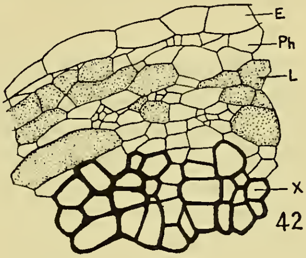

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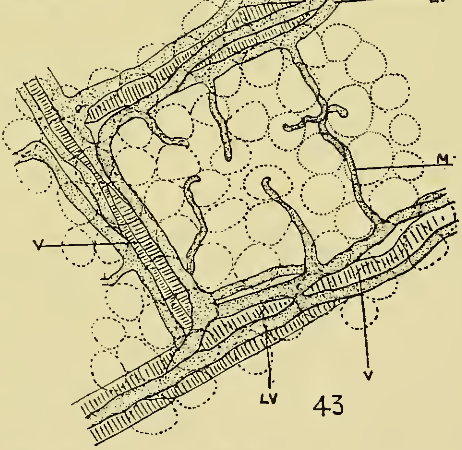

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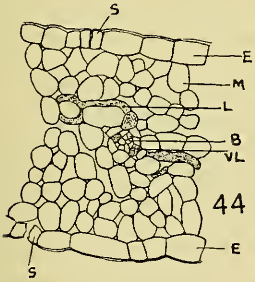

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Text-fig. 41.—Part of a transverse section of a young aerial stem of *L. gibbosa*. Cu, cuticle; Ep, epidermis; C, cortex; CL, cortical latex tubes; E, endodermis; L, latex tubes in the phloem; Ph, phloem; Ca, cambium. × 52.

Text-fig. 42.—Part of a transverse section of an old main root of *L. dentata* showing portion of the stele. E, endodermis somewhat stretched by the formation of secondary xylem; Ph, phloem; L, latex tubes which have increased considerably in size and number (cf. Text-fig. 19); X, secondary xylem. × 150.

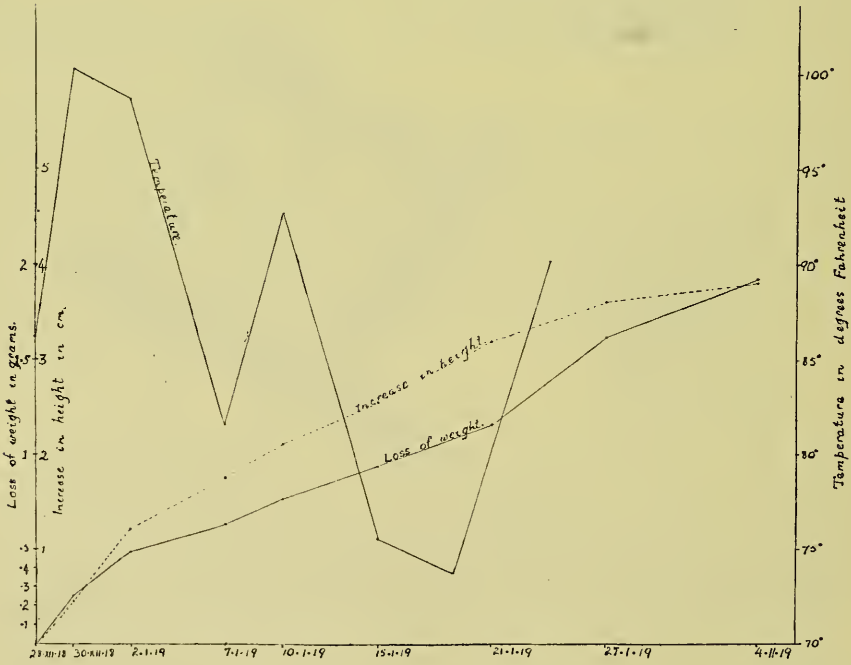
Text-fig. 43.—Surface view of part of a leaf of *L. dentata* as seen after soaking in 5% caustic potash for five days, showing the course of the latex tubes in the mesophyll. V, veins; LV, latex tubes accompanying the veins; M, short branches of the latex tubes which ramify through the mesophyll. × 150.

Text-fig. 44.—Part of a transverse section of a young leaf of *L. dentata*. S, stomate; E, epidermis; M, mesophyll; B, vascular bundle; VL, latex tubes accompanying the vascular bundle; L, latex tubes in the mesophyll. × 150.

walls. Text-figure 42 shows part of a transverse section of an old root; when this is compared with Text-figure 19, it is evident that the latex system has enlarged very considerably. Similarly in the young stem (Text-figure 37) the latex tubes are small and not very numerous, but in a section of an older stem they are much enlarged (Text-fig. 39). In transverse sections of the aerial stems of both *L. dentata* (Text-fig. 38) and *L. gibbosa* (Text-fig. 41) the latex cells can be seen ramifying through the cortex. They are also abundantly present in the leaf, several vessels accompanying each vein, and giving rise to branches which follow a sinuous course through the mesophyll (Text-figs. 43, 44). Latex vessels are also present in all parts of the flower.

General Biology, Experiments and Observations.

Experiments were made by Miss Rennie during the summers of 1917–18 and 1918–19 on the growth of shoots of *L. gibbosa* when dug up and allowed to dry. Measurements were made at intervals of the length of the plants from a marked point on the stem to the apex, and each plant was weighed. Comparable results were obtained in both cases. The data obtained from the 1918–19 experiment are combined in the accompanying graph.



Graph showing loss of weight and increase in height. Average loss of weight (5 plants) 1·17 grm. in 38 days; max. loss 1·93 grm.; min. loss 0·75 grm. Average increase in height (5 plants) 3·85 cm. in 38 days; max. increase 6·4 cm.; min. increase 2·8 cm.

Five plants were weighed and measured and the results averaged. The curves representing increase in height and loss in weight are very similar; the loss in weight is at first fairly sharp and then becomes less, rising again towards the end of the experiment. After an initial spurt, increase in height became gradually less till it ceased with the opening of the last bud at the end of the experiment.

There is no evidence that either loss of weight or increase in height was influenced, during this period, to any marked extent by the external environment, since, while the experiment was in progress, there was a period of heat with a maximum shade temperature of 106·8° on 5th January and two spells of fairly cool weather, which caused no noticeable variation.

In contrast to this behaviour, plants of *L. dentata* were found to wilt in two or three days if the soil in which they were growing were allowed to dry out, and plants which were dug up and allowed to dry wilted and died at once. Even plants with large underground stems could not withstand a much longer period of dryness.

The reserve food in the fungus in growing roots.—The droplets of reserve food in the fungal hyphae during the fungal enlargement period and the droplets which appear in the cortical cells of the root during the subsequent period of fungal depletion stained at first dark brown with 1% osmic acid. When hand sections so stained were mounted in glycerine jelly, in a few days the brown colour diffused out from the sections leaving the droplets stained a dense black. In material which was fixed in Flemming's fixative and embedded in paraffin, the droplets appear black, the brown staining material having evidently been washed out, probably in alcohol. This reserve food is entirely soluble in chloroform, and stains in Sudan III, and in sections of fresh material, collects in droplets around the section. It is probably an oily material, mixed with a diffusible alcohol-soluble proteinaceous substance.

Reserve food in the subterranean stem of L. dentata.—Positive tests for sugar in the cortex of subterranean seedlings of *L. dentata* were obtained by the alpha naphthol test, and by Mayer's method (Haas and Hill, 1928). The Osazone test (Mangham, 1915) showed more clearly in which part the sugars were most abundant, and also gave some indication of the type of sugar involved. In very young stems the sugar is distributed throughout the cortex fairly evenly. In about six weeks after treatment of sections of young stems by this method the sugar commences to crystallize in large clusters from droplets of a golden-brown liquid, and from the shape of the crystals and their manner of formation it is probable that the sugar involved is maltose.

In older stems, the sugars become restricted to the middle and inner cortex, and at the same time become abundant in the latex. In still older stems no sign of sugars can be found in the cortex, and they finally become much less abundant in the latex also.

The staining reactions of the latex with 1% osmic acid and with potassium iodide-iodine suggest the presence of oily and proteinaceous substances but no starch. Partial solubility of the latex in 5% alcoholic solution of tartaric acid (Haas and Hill, 1928) suggests the presence of an alkaloid.

Discussion.

a. Germination and the Young Seedling.

The seed before germination is packed with oil, but even so, so small a seed could not give rise to a very large seedling unaided by photosynthesis or some other means, and, as we saw, a relatively enormous subterranean seedling develops from it. It seems, therefore, undoubted that the fungus which is invariably associated with the seedling must provide the necessary food for such growth, and that we must regard *Lobelia dentata* and *Lobelia gibbosa* as holoparasites on the fungus, at least in the subterranean stage of their growth.

The following is a description of what is believed to occur in germination. It has been deduced from an examination of fairly large seedlings, and must be confirmed by experiment before it can be entirely accepted. The embryo starts to grow at the expense of the endosperm, producing a small, probably undifferentiated, protocorm-like structure; the testa becomes thrown off and the endosperm cells remain for a time as broken fragments on the outside. This is a critical period and probably the time when most loss of life occurs. The plant has used up all its reserve food and is still far below the surface of the soil, with no chance of becoming self-supporting. It is therefore imperative that an association with the fungus should take place. This appears to come about as follows: in the soil there are present rhizomorph-like strands of fungal hyphae; one of these grows into contact with a young *Lobelia* embryo, the strand spreads out over the surface of the embryo, forming a practically continuous mat several cells deep; it is evidently attracted there by some chemical substance. Wedges of mycelium are pushed in between the epidermal cells and into the cortex, remaining, however, strictly intercellular. The young plant is able to extract carbohydrate and nitrogenous food from the fungus, and enlarges rapidly so that the cells of the cortex become much stretched tangentially. A shoot then grows upwards bearing rudimentary leaves, and a root grows downwards, which is from the beginning infected by the fungus.

It seems rather extraordinary that germination should as a rule take place so far below the surface of the soil. Such small seeds, however, might be fairly easily washed down into crevices and covered up; a rather favourite place for germination is in accumulations of soil washed up against rocks or the bases of tree trunks in which seeds could readily become buried. Possibly also, the fungus necessary for germination is not present in the upper layers of the soil.

The form of seedlings which commence growth in heavy soil can probably be ascribed to the physical conditions of their surroundings. Such seedlings would naturally find a greater barrier to their upward growth than seedlings growing in light sandy soil. It is therefore reasonable to expect that they would take longer to reach the surface, and so there would be a longer period for food to accumulate in them. This would account for their large size and might possibly induce the formation of branches. Especially hard patches in the soil or fragments of stone would cause the seedling to become twisted.

b. Mycorrhiza.

A number of interesting points in connection with the mycorrhiza have emerged during the investigation of *L. gibbosa* and *L. dentata.*

It appears to be the first instance noted of the obligate association of an annual herb with a mycorrhizal fungus, though obligate associations of annual parasitic plants with their hosts are not uncommon. It is considered justifiable to regard these two species of *Lobelia* as obligate parasites on the fungus, because germination invariably takes place at such depths below the surface of the soil as to make it impossible for the young seedling to become self-supporting at once, and because all root systems examined showed the presence of the fungus.

A comparison of this type of mycorrhiza with other known types (Rayner, 1927) makes it evident that it cannot be grouped satisfactorily with any of them. First we have a two-phase mycorrhiza in *Lobelia*; the root system of a plant is infected from the beginning of its growth by the fungus which invades the hypocotyl, this fungus keeping pace with the growing root by invading the newly-formed tissues; it then builds up a reserve of food material within its cells and later loses this to the cells of the cortex of the root; but the root system is also subject to more or less numerous local reinfections from the soil by isolated rhizomorphs of the same fungus.

Secondly, the behaviour of both sets of mycelium within the root is unusual in that the hyphae are at all times strictly intercellular. Thirdly, the mechanism of extraction of food materials by an intercellular mycelium from the cells between which it grows is extraordinary in a mycorrhiza, and rare even in fungal parasites of higher plants. The ensuing extraction of food from the fungus by the cells of the cortex is no less unusual. It seems to be due to changes in relative permeability on the part of the cells of both the mycelium and the root cortex. In the first place food material accumulates in the hyphae, some of it possibly at the expense of the root. Subsequently these food materials disappear from the fungus and at the same time reappear as minute drops in the peripheral cytoplasm of the neighbouring cortical cells. The osmotic exchanges involved must take place through two sets of semipermeable membranes and the adjacent cell walls, those of the fungus and the cortical cells. But the nature of the changes which take place in the cells of these two organisms is not known, nor even the condition in which the food stuffs are transferred.

There is not a great deal of similarity between ectotrophic mycorrhiza and the mycorrhiza of *Lobelia*. Ectotrophic fungi, though mainly intercellular, are occasionally intracellular; they are usually limited to the outer cortex of the root and the mycelium forms a thick mat over the surface of the root and penetrates into it at all points; whereas in the case of the *Lobelia* mycorrhiza, each rhizomorph has a single point of entry into the root, and the mycelium penetrates throughout the cortex.

There is even less in common between the *Lobelia* mycorrhiza and the endotrophic types which produce "arbuscules", for in this case also the arbuscules are intracellular and become digested by the cells they invade.

In the case of the orchid mycorrhiza also the behaviour of the fungus within the plant has no similarity to that of the *Lobelia* mycorrhiza. In the orchid the fungus is entirely intracellular and forms clumps of hyphae within the cells and these are digested later by the higher plant.

There is some similarity between the germination of orchid seeds and what is believed to be the case in *Lobelia*. In both cases fungal infection comes from the soil, in orchids it is obligate except under artificial conditions, and in *Lobelia* successful germination in the field seems undoubtedly to depend on fungal infection. Whether the similarity is more than superficial cannot be determined until *Lobelia* seedlings have been cultivated under controlled laboratory conditions. It has been shown that some orchids are entirely free of mycorrhizal fungus in the adult condition and that they evidently behave as autotrophic plants. For *Lobelia* also there is evidence that, once able to conduct photosynthesis, the plant, if favourably situated, can support itself.

The invasion of roots of *L. gibbosa* and *L. dentata* by separate strands of fungal hyphae finds its nearest comparison in the mycorrhiza of the orchid *Gastrodia elata*, which is described by Kusano (1911) as forming an obligate association with the rhizomorphs of the fungus *Armillaria mellea*, but the likeness ceases with the external appearance, since *Armillaria* commences by making a parasitic attack which is countered by the orchid, and its hyphae become intracellular and are ultimately digested by the cells of the orchid.

It may be of some importance that the nuclei of the cells of the cortex of the root of *Lobelia* never at any stage become enlarged and distorted as is commonly the case in the development of other mycorrhizal associations.

It must be admitted that the method of nutrition of *L. gibbosa* and *L. dentata* is not yet fully elucidated, nor can it be until plants can be grown from seed under controlled conditions.

Without evidence to the contrary it seems as if the subterranean seedling must rely for its entire food supply on those fungal strands from the soil which make connections with its roots, since the hyphae which grow down from the hypocotyl within the root have no direct connection with the outside medium. But a careful examination of numerous roots showed that though the external strands were occasionally very abundant, they were often very infrequent. Though they may be present in sufficient numbers to supply the growing plant with all its food requirements, it is not considered justifiable to assume that this is always the case. The function of the fungus which develops within the root is at present obscure. It is possible that it may utilize soluble organic substances absorbed in the soil solution by the root, and convert these into a material which is available to the plant. Unless this is the case, this phase of the fungus, once the seed has germinated, seems to be of the nature of a parasite which is kept in check by the higher plant.

c. Latex System.

Latex systems of plants have been classified according to their origin into two groups, latex cells which grow through the plant body after the manner of fungal cells and are non-septate throughout their length, and latex vessels which result from the breaking down of end walls between superimposed rows of cells. In *Lobelia* the latex system combines features of both types, the main latex tubes in the phloem belong to the second category, but the irregular anastomoses, and the ramifying branches in the cortex are almost certainly due to outgrowths of parts of the walls of the main latex vessels, and therefore come into the first class. This is also probably the case for the finer tubes in the mesophyll of the leaf, whereas the main latex tubes of the leaf which accompany the veins, not infrequently show traces of transverse walls in sections of very young leaves.

The function of the latex system in the roots and stem seems to be undoubtedly that of conduction; in support of this there is the fact that as the fungus at the root tips becomes depleted, the latex tubes become filled with food material. Also, in the underground stem, sugar gradually disappears from the cortex and at the same time the latex becomes more and more abundant. Both in the root and in the stem the number and size of the sieve tubes are very insignificant. It seems also to be important that the chlorophyll bearing tissue of both stem and leaves is intersected and tapped by latex tubes very abundantly; here it seems very likely that the latex tubes serve to carry away the products of photosynthesis.

d. General Biology.

Whilst the seedlings of *Lobelia* are subterranean, there is no doubt that they are wholly dependent on the fungus associated with their roots, both for nitrogenous and carbohydrate foods; but as soon as the plant comes above ground it is at least partly self-supporting, since it develops an abundance of chlorophyll and occasional thin absorbing roots.

Though the method of nutrition of the two species is fundamentally the same, there are important differences in their behaviour. *L. gibbosa* has been shown to behave as a succulent in that it continues to grow in length and open flowers, at the same time slowly losing weight, even when removed from the soil and supplied with no water. *L. dentata*, on the other hand, is a much less robust plant, and in spite of the fact that it frequently has a great length of subterranean stem, which in the young plant may contain considerable quantities of water, this species has none of the characteristics of a succulent.

In the case of *L. dentata*, though the development of an aerial shoot is of advantage in that it ensures a more reliable source of food, it introduces a danger to which the subterranean seedling was not subjected, namely, that of transpiration. It is very evident that *L. dentata* is unable to cope with conditions of rapid transpiration; plants growing in open sunny situations are practically always much dwarfed as compared with shaded plants. This is very probably the direct result of the structure of their root system, which even a superficial examination will show to be eminently unsuited for rapid water absorption, being devoid of root hairs and composed of roots which are too thick to form close contact with soil particles. This explains why plants of *L. dentata* show symptoms of water shortage in positions where other plants, whose root systems are more efficient in the absorption of water, can flourish. It also explains why dwarfed plants whose flowering period was over, when transplanted into pots and abundantly supplied with water, develop fresh lateral shoots and new roots.

Summary.

Lobelia gibbosa and *Lobelia dentata* are annual herbaceous plants whose manner of growth shows several points of interest.

L. gibbosa is widely distributed throughout Australia. Its average height is about 47 cm., but specimens growing in exposed positions are usually much shorter. The above-ground stem is succulent. At the time of flowering the root and underground part of the stem and the lower leaves are withering or dead, the plant evidently living on the stored materials in the stem.

L. dentata is limited to the coast and adjacent highlands of New South Wales and southern Queensland. It is on the whole a smaller and less robust plant than *L. gibbosa*, and its aerial stem is not succulent. The underground part of the stem may be 15 cm. or more long, in contrast with *L. gibbosa*, whose underground stem averages about 5 cm. in length. When the plant is young the cortex of the subterranean part of the stem is wide and succulent and a good deal of sugar is present. As the plant increases in size the sugar becomes transferred to the latex vessels and transported away, and the cortex shrinks, evidently losing

water as well as sugar. As is the case with *L. gibbosa*, plants growing in exposed positions are much smaller than more favoured plants.

In both species germination usually takes place at a distance below the surface of the soil, and at first the seedling is entirely subterranean. Successful germination, therefore, depends on an early infection by a fungus, since the seeds are very small and do not contain sufficient reserve food material to provide for much growth.

Strands of fungal hyphae are always found associated with the hypocotyl region of young seedlings; they spread out forming a mat over its surface and in places penetrate into the cortex by pushing down between the epidermal cells. In the cortex they remain strictly intercellular, and in transverse section are seen to be most abundant in the outer cortex, probably because the hyphae which penetrate into the inner cortex are quickly crushed out. This intercellular fungus evidently infects the young primary root as soon as it commences growth, and keeps pace with it as it grows, also infecting all lateral roots and their branches.

The apex of a young growing root is free from fungal hyphae. Behind this area three stages in the association of the fungus and the cells of the root can be distinguished:

1. *The period of fungal invasion.*—In a longitudinal section near to the apex of a young growing root, the invading hyphae can be seen growing down between the cortical cells, and are evenly distributed throughout the whole cortex. At this time the fungal hypae are narrow and densely protoplasmic.

2. *The period of fungal enlargement.*—Sections taken successively further and further from the apex of a young growing root show that the fungus becomes concentrated in the middle cortex forming a definite fungal zone. In this region the fungal cells become much enlarged by the accumulation of droplets of reserve food of an oily nature within them, and the cortical cells become forced apart. The fungal hyphae in the inner and outer cortex are not only less numerous, but also thinner and contain less reserve food.

3. *The period of fungal depletion.*—Sections cut behind the region showing fungal enlargement show that changes take place in the fungal zone resulting in the disappearance of all reserve food from the fungal hyphae and the accumulation in the peripheral cytoplasm of the neighbouring cells of very numerous small droplets of reserve food with similar staining reaction. The depletion of the fungus commences in the inner fungal zone and continues outward until there is no reserve food remaining in the fungus. During this period the cells of the outer cortex increase in size, gradually crushing the fungus till none remains except a few living threads in the outer cortex. Food material is evidently extracted by the fungus from the cortical cells and later by the cortical cells from the fungus by osmosis, since no signs of haustorial organs could be found. As the food material is extracted from the fungus it is transported across the cortex to the latex vessels and thence to the growing stem.

Strands of fungal hyphae similar to those associated with the hypocotyl of seedlings are also found associated with the roots. The outer part of the strand consists of a number of narrow thick-walled hyphae, somewhat intertwined but with a general longitudinal course. These are closely bound together so as to form a continuous tissue protecting the core of the strand which consists of one or more wide, thin-walled, densely protoplasmic hyphae, whose course is strictly longitudinal.

Where these strands grow into contact with a root of *Lobelia* the outer layer spreads out over the surface forming a mat which may be extensive or very narrow. From the core a strand of hyphae grows down into the cortex of the root, penetrating between the epidermal cells and growing through the intercellular spaces of the cortex.

The main points of importance of the latex system are described. In the roots and underground stem the latex system is confined to the phloem and consists of large vessels with numerous anastomosing branches. The vessels result from the breaking down of end walls between rows of superimposed cells, and the branches are probably prolongations of the cell wall. In the aerial stem the main part of the latex is in the phloem, and is similar to that of the underground stem, but a characteristic feature is that branches grow out from the main vessels, and penetrate between the cells of the endodermis and ramify between the chlorophyll-bearing cells composing the cortex. In the leaf several latex vessels accompany each vein and numerous branches grow out between the mesophyll cells. Latex vessels are also present in all parts of the flower. It is concluded that in *L. gibbosa* and *L. dentata* the latex system functions mainly in transport of food materials.

L. gibbosa behaves as a succulent when deprived of water, but *L. dentata* under similar conditions dies at once in spite of the considerable development of the cortex in the underground stem, which in the young plant contains much water.

From its general structure the root system of the two species appears to be an inefficient organ for the rapid absorption of water, and it is for this reason that plants in fairly exposed situations do not flourish, though other plants whose root systems are not modified, grow well and show no signs of water shortage under the same conditions.

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